The role of neuronal excitability, allocation to an engram and memory linking in the behavioral generation of a false memory in mice

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ABSTRACT

Memory is a constructive, not reproductive, process that is prone to errors. Errors in memory, though, may originate from normally adaptive memory processes. At the extreme of memory distortion is falsely "re-membering" an event that did not occur. False memories are well-studied in cognitive psychology, but have received relatively less attention in neuroscience. Here, we took advantage of mechanistic insights into how neurons are allocated or recruited into an engram (memory trace) to generate a false memory in mice using only behavioral manipulations. At the time of an event, neurons compete for allocation to an engram supporting the memory for this event; neurons with higher excitability win this competition (Han et al., 2007). Even after the event, these allocated "engram neurons" remain temporarily (~6 h) more excitable than neighboring neurons. Should a similar event occur in this 6 h period of heightened engram neuron excitability, an overlapping po-pulation of neurons will be co-allocated to this second engram, which serves to functionally link the two memories (Rashid et al., 2016). Here, we applied this principle of co-allocation and found that mice develop a false fear memory to a neutral stimulus if exposed to this stimulus shortly (3 h), but not a longer time (24 h), after cued fear conditioning. Similar to co-allocation, the generation of this false memory depended on the post-training excitability of engram neurons such that these neurons remained more excitable during exposure to the neutral stimulus at 3 h but not 24 h. Optogenetically silencing engram neurons 3 h after cued fear conditioning impaired formation of a false fear memory to the neutral stimulus, while optogenetically activating engram neurons 24 h after cued fear conditioning created a false fear memory. These results suggest that some false memories may originate from normally adaptive mnemonic processes such as neuronal excitability-dependent allocation and memory linking.

1. Introduction

Our memories help define who we are. Mnemonic processes allow us to recall the past, function in the present and envision the future. Yet memories are not stored and recalled as exact copies of our experiences. Schacter (1999, in press) identified seven "sins" of memory, including the sins of forgetting (i.e., transience, absent-mindedness and blocking), memory persistence (i.e., intrusive, unwanted memories), and memory distortion (i.e., misattribution, suggestibility and bias). Mis-remembering where we put our keys is an everyday example of a memory distortion that may produce relatively small consequences. However, at the extreme end, are memory distortions that lead to more serious consequences such as when eyewitnesses misidentify innocent individuals (Laney & Loftus, 2013; Wells & Olson, 2003) or when in-accurate memories of childhood sexual abuse are "recovered" (Bremner, Shobe, & Kihlstrom, 2000; McNally & Geraerts, 2009). Dif-ferent memory distortions may be mediated by different neural me-chanisms.

In the lab, human memory may be experimentally distorted via several types of interventions (Brainerd & Reyna, 2005; Gallo, 2006;

Roediger & McDermott, 2000; Slotnick & Schacter, 2004). Post-event misinformation, for instance, can be used to contaminate the memory of a previous event (Loftus, 2005). In a classic study, misleading post-event information was often incorporated into subsequent event reports (Loftus, Miller, & Burns, 1978). Moreover, entirely false memories of events that never occurred may also be "implanted" in human participants (Loftus, 2003; Shaw & Porter, 2015). Human neuroimaging studies are beginning to characterize large-scale neural processes associated with memory distortions (Kurkela & Dennis, 2016; Schacter, Carpenter, Devitt, & Thakral, in press). By comparison, the neurobiological mechanisms underlying memory distortions have received far less attention. While optogenetic stimulation of tagged neural circuits has been used to create a false memory in mice (Garner et al., 2012; Ramirez et al., 2013; Vetere et al., 2019; Yokose et al., 2017), we are unaware of studies using purely behavioral manipulations to create a false memory for a discrete cue in rodents.

Schacter (1999; Schacter, Guerin, & St Jacques, 2011) and others (Howe, 2011; Loftus, 2005) suggest that some memory distortions reflect normally advantageous functional memory processes gone awry. To examine this hypothesis, we took advantage of recent findings in mice indicating that two similar events that occur in close temporal proximity can become functionally linked by virtue of neuronal co-allocation to overlapping engrams (Rashid et al., 2016). Here, we investigated whether neuronal co-allocation, a fundamental mechanism mediating the adaptive mnemonic process of memory linking, can be co-opted to create an entirely false cued fear memory in mice using purely behavioral procedures.

1.1. Neuronal allocation and memory linking (Fig. 1)

The lateral nucleus of the amygdala (LA) is known to be a critical brain region involved in discrete cue fear conditioning (Davis, 1992; Josselyn et al., 2001; LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; Maren & Fanselow, 1996; Maren, 2005). Results from many experiments suggest that within a given brain region, such as the LA, eligible neurons compete for allocation to an engram (or memory trace) supporting a given memory. Moreover, neurons with relatively increased excitability at the time of an event "win" this competition to become "engram neurons" (neurons that are critical components of what is likely a larger engram and are required for subsequent recall of that particular memory) (Cai et al., 2016; Gouty-Colomer et al., 2015; Han et al., 2007; Hsiang et al., 2014; Park et al., 2020; Park et al., 2016; Rashid et al., 2016; Yiu et al., 2014; Zhou et al., 2009). After a training event, allocated engram neurons remain temporarily (~6 h in the LA) more excitable than neighboring neurons (Cai et al., 2016; Pignatelli et al., 2019; Rashid et al., 2016). The enhanced post-training excitability of engram neurons has important implications for neuronal engram allocation to subsequent events. For instance, if a similar event occurs during the time of engram neuron increased excitability (within ~6 h of Event1), these engram neurons (or a subset thereof) supporting Event1 are also allocated to an engram supporting Event2, in a process termed co-allocation. By virtue of co-allocation to overlapping engrams, the memories for these two events become linked (Fig. 1A).

At later time points after an event (> 6h-24 h in the LA), homeostatic processes may decrease the excitability of Event1 engram neurons relative to their neighbors, such that these neurons become "refractory" to subsequent allocation. Should Event2 occur in this post-training time window, Event1 engram neurons would be less excitable than their neighbors and a novel population of relatively more excitable neurons would be allocated to an engram supporting Event2. This process is termed dis-allocation. Event1 and Event2 would be not be linked, but remembered separately (Rashid et al., 2016), similar to pattern separation (Fig. 1A).

Importantly, in our previous co-allocation and dis-allocation experiments (Rashid et al., 2016), Event1 and Event2 were cued fear conditioning (in which an initially motivationally neutral conditioned

stimulus (CS, typically a tone) was paired with an aversive unconditioned stimulus (US, a footshock)). Here we asked whether a similar neuronal excitability-based co-allocation process could be "hijacked" to generate a false cued fear memory. In the present experiments, we trained mice with cued fear conditioning (Event1) as before, but presented a motivationally-neutral tone (CS2) alone (rather than a tone-footshock Event2) either 3 h or 24 h after auditory fear conditioning (Fig. 1B).

2. Methods & materials

2.1. Mice

Adult (> 8 weeks of age) male and female F1 hybrid (C57BL/6NTac × 129S6/SvEvTac) wild-type mice were used. All experimental procedures were performed in accordance with policies of the NIH Guidelines on the Care and Use of Laboratory Animals and Canadian Council on Animal Care (CCAC) and approved by the Hospital for Sick Children's Animal Care and Use Committee.

2.2. Gaining access to engram neurons

In "engram tag-and-manipulate" strategies inducible immediate early gene promoters [using minimal Fos, Arc promoters or artificial promoters coupled to tTA or CreER[T2] systems (Denny et al., 2014; Garner et al., 2012; Guenthner, Miyamichi, Yang, Heller, & Luo, 2013; Kawashima et al., 2013; Liu et al., 2012; Sørensen et al., 2016)] are used to drive the expression of optogenetic or chemogenetic constructs in neurons active during a training even to allow subsequent manipulation of these cells. As this strategy requires transcription of the optogenetic construct (and therefore require several hours after a training event for sufficient transgene expression), this strategy is not ideal for the current experiments in which we manipulate engram neurons 3 h post-training. Therefore, we used an "allocate-and-manipulate" strategy (Frankland, Josselyn, & Köhler, 2019; Josselyn, Köhler, & Frankland, 2015) to bias the allocation of neurons expressing optogenetic constructs before training and manipulated the activity of these engram neurons in the hours after training.

A viral vector expressing two optogenetic constructs responsive to two non-overlapping wavelengths of light (NpACY) was central to this "allocate-and-manipulate" strategy (Fig. 4A) (Rashid et al., 2016; Stahlberg et al., 2019; Zhang, Aravanis, Adamantidis, de Lecea, & Deisseroth, 2007). NpACY expresses both the blue light (BL)-sensitive excitatory opsin channelrhodopsin-2 [ChR2-H134R; fused to enhanced yellow fluorescent protein (eYFP)] and the red light (RL)-sensitive inhibitory opsin halorhodopsin (eNpHR3.0)(Stahlberg et al., 2019; Zhang et al., 2007).

To bias the allocation of infected LA neurons into the engram, we photostimulated with blue light (BL, 473 nm) to activate ChR2 and increase the excitability of infected neurons (Fig. 4B). After training, this same population of neurons could either be artificially excited with BL (to activate ChR2) or inhibited with red light (RL, 660 nm to activate eNpHR3.0) (Fig. 4B). Previous whole-cell current-clamp experiments of hippocampal neurons verified the activation spectra for ChR2 and NpHR are separable by ~100 nm, allowing distinct activation of each opsin (Zhang et al., 2007). Moreover, BL (488 nm) increases, while RL (594 nm, 639 nm) decreases, the activity of cells expressing this construct, with minimal cross-talk (Rashid et al., 2016; Stahlberg et al., 2019).

2.3. Viral vectors

We used several viral vectors.

[1] HSV-NpACY. We used a replication-defective herpes simplex virus (HSV) vector expressing two optogenetic constructs: ChR2 and eNpHR3.0 (Fig. 4A). Infected neurons could be visualized using YFP.

A

Neuronal allocation and memory linking (derived from Rashid et al., 2016)
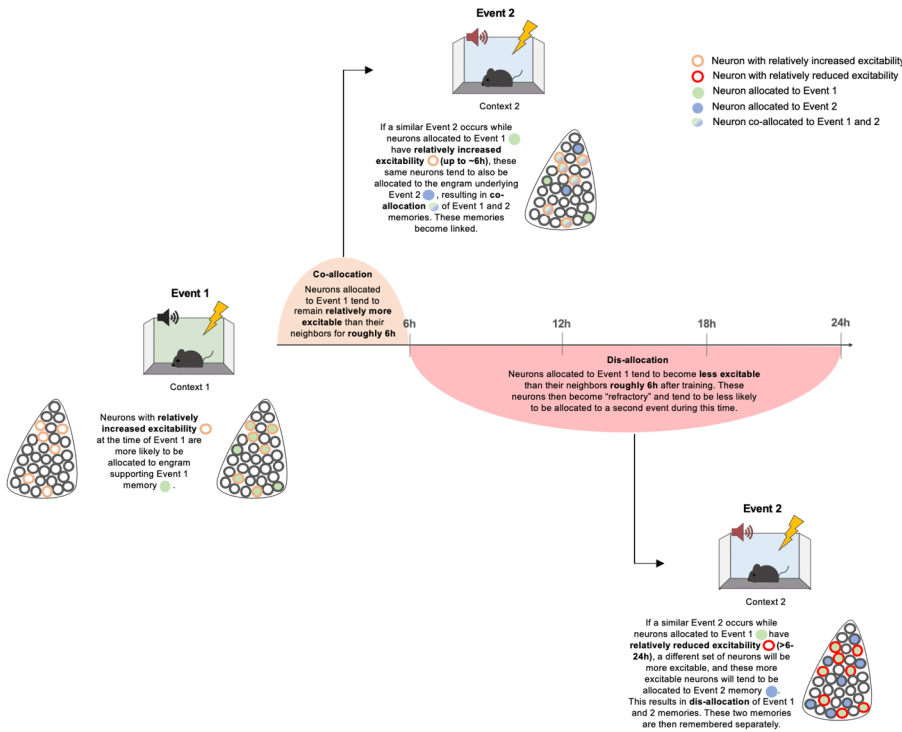
B

Using neuronal allocation to generate a false memory: a hypothesis.
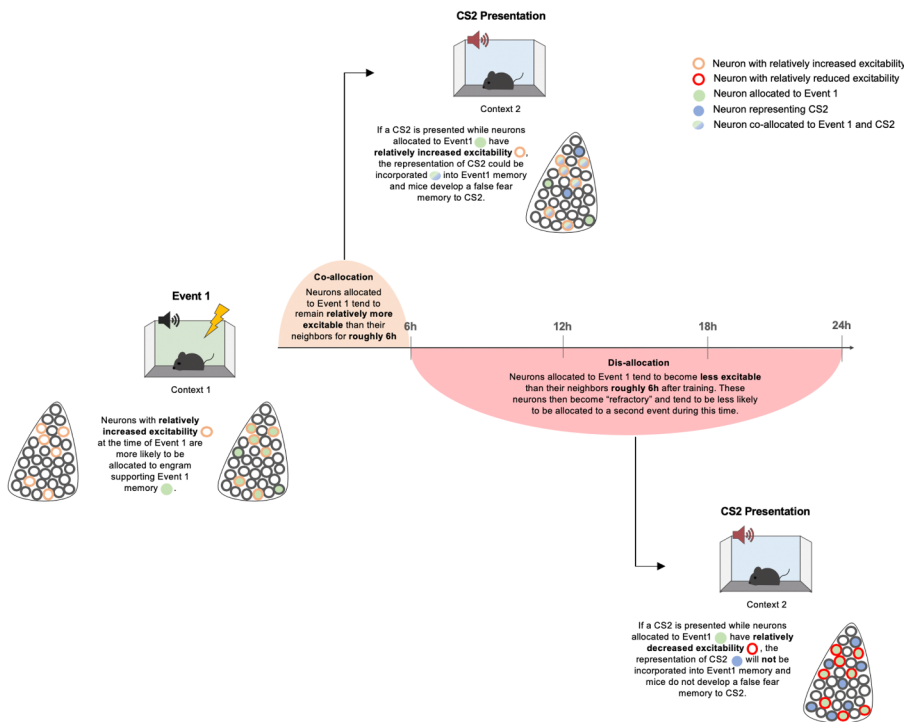

Fig. 1. Depiction of neuronal allocation, memory linking and false memory creation hypothesis. (A) Memories of two similar events occurring in close temporal proximity become linked by virtue of neuronal allocation to overlapping engrams (termed co-allocation) (from Rashid et al., 2016). Lateral amygdala (LA) neurons with relatively increased excitability (orange outline) during Event1 (CS1 + footshock) become allocated to engram supporting Event1 memory (neurons filled green). These allocated engram neurons remain relatively more excitable than neighbors for ~6 h post-training (orange outline), before becoming less excitable than their neighbors (~24 h post-training, red outline). If a similar Event2 (CS2 + footshock) occurs shortly after Event1 (within 6 h time-period of heightened engram neuron excitability), Event1 engram neurons also become allocated to Event2 engram (co-allocation, neurons filled with blue + green), linking the memories for Event1 and Event2. However, if Event2 occurs > 6 h after Event1, a distinct population of neurons is allocated to Event2 engram (blue neurons), the engrams are dis-allocated and the two memories are remembered separately. (B). We examined whether neuronal allocation mechanisms underlying memory linking could be hijacked to create a false fear memory. Either 3 h or 24 h post-cued fear conditioning (CS1 + footshock), mice were presented with motivationally-neutral tone CS (CS2). We examined whether a false fear memory to this tone CS was created in the 3 h, but not 24 h, group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

For Fig. 6, we used an HSV expressing ChR2 fused to mCherry. HSV expressing fluorophore alone (e.g., TdTomato) served as a control.

In all HSV viruses, transgene expression was driven by the IE 4/5 promoter (Rashid et al., 2016). HSV-derived particles were packaged in-house to titers > 1×10^8 infectious units/ml (Han et al., 2007). When microinjected into the LA, HSV randomly infects approximately 10–15% of principal (excitatory) neurons (Fig. 5C, Fig. 6C) not interneurons (Yiu et al., 2014) and reaches maximal expression levels 2–4 d post-injection (Barrot et al., 2002; Carlezon, Nestler, & Neve, 2000; Carlezon & Neve, 2003; Neve, Neve, Nestler, & Carlezon, 2005; Park et al., 2020).

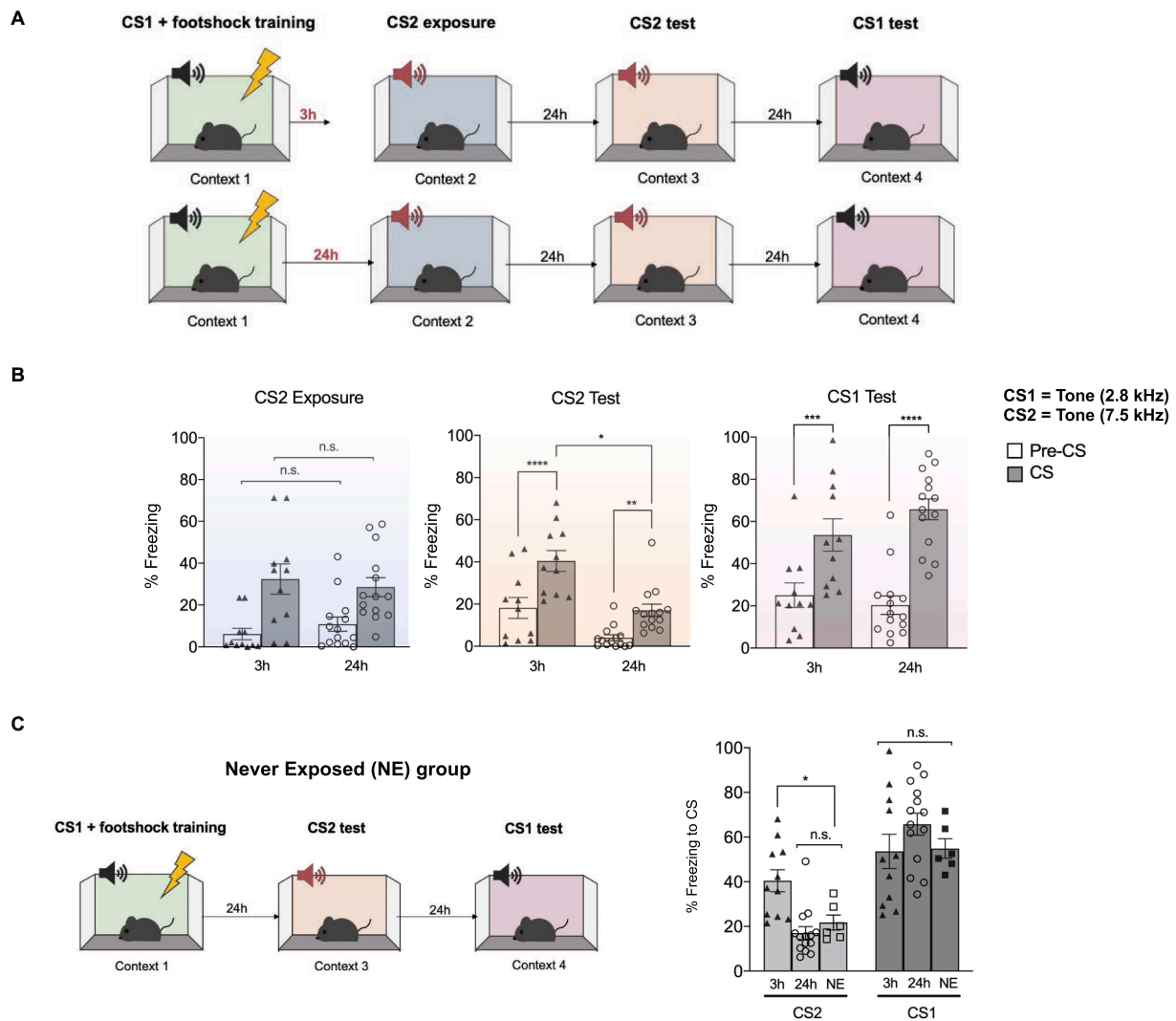[2] AAR-RAM-GFP. To examine whether optogenetic "priming" of LA

Fig. 2. Behavioral generation of a "false fear memory" in mice using two auditory stimuli. (A) 3 h or 24 h post-auditory fear conditioning (CS1 + footshock), a motivationally-neutral CS2 tone was presented in a new context (without footshock). Freezing to CS1 (real conditioned fear memory) and CS2 (false memory) subsequently tested in novel contexts. (B) Similar freezing to novel auditory CS2 (CS2 Exposure) presented either 3 h (n = 11) or 24 h (n = 14) post-auditory CS1 + footshock fear conditioning. During memory tests, 3 h mice froze more to CS2 than 24 h mice, but both groups froze at similar levels to CS1. (C) During a memory test, mice presented with CS2 24 h after fear conditioning froze at similar levels to mice first exposed to CS2 during the memory test (never exposed, NE; n = 6). All groups froze similarly to original CS1. Data represent mean ± SEM unless otherwise specified.

neurons before auditory fear conditioning preferentially allocates these neurons to an engram supporting the auditory fear memory, we used the robust activity marking (RAM) "engram tagging" system (Sørensen et al., 2016). The AAV-RAM-GFP viral vector tags (with GFP) active neurons (via a synthetic activity-regulated promoter, P_{RAM}, composed of minimal AP-1, Fos and Npas4 promoter sequences) in a temporally-specific fashion [via a doxycycline (DOX)-dependent modified Tet-Off system]. pAAV-RAM-d2TTA::TRE-EGFP-WPREpA was a gift from Yingxi Lin (Addgene plasmid # 84469; http://n2t.net/addgene:84469; RRID:Addgene_84469). AAVs (DJ serotype) were generated in HEK293T cells with the AAV-DJ Helper Free Packaging System (Cell Biolabs, Inc. # VPK-400-DJ) using the protocol suggested by the manufacturer. Viral particles were purified using Virabind AAV Purification Kit (Cell Biolabs, Inc. # VPK-140), yielding final viral titers of approximately 10^{11}/ml.

2.4. Surgery

Mice were pre-treated with atropine sulfate (0.1 mg/kg, i.p.), anesthetized with chloral hydrate (400 mg/kg, i.p.) or isoflurane-oxygen mix (3% isoflurane for initial induction and 1–2.5% through nose cone thereafter), administered meloxicam (2 mg/kg, s.c.) for analgesia, and placed in a stereotax. HSV vectors were infused bilaterally (2 μL/side, flow rate 0.12 μL/min) into the LA (AP: −1.3 mm, ML: ± 3.4 mm, DV: −4.8 mm relative to bregma) and optical fibers implanted above the LA (AP: −1.3 mm; ML: ± 3.4 mm, DV: −4.3 mm). Behavioral experiments were conducted 3–4 d post-surgery during maximal transgene expression. In Fig. 6, mice were microinjected with AAV-RAM-GFP microinjections (1 μL/side, flow rate 0.12 μL/min).

2.5. Behavioral procedures

Tone cued fear conditioning. Mice were placed in a conditioning chamber and, 2-min later, presented with CS1 (conditioned stimulus 1, auditory tone; 2.8 kHz tone, 85 dB, 30 s) that co-terminated with a footshock (0.5 mA, 2 s). Mice were returned to the home-cage 30 s later.

Light cued fear conditioning (Fig. 3A). The same procedure as above was used except the tone-CS1 was replaced with a fluorescent light (visual CS1; luminous emittance 500–600 lx, 30 s). The light CS1 co-terminated with a footshock (0.5 mA, 2 s). Mice received two light-
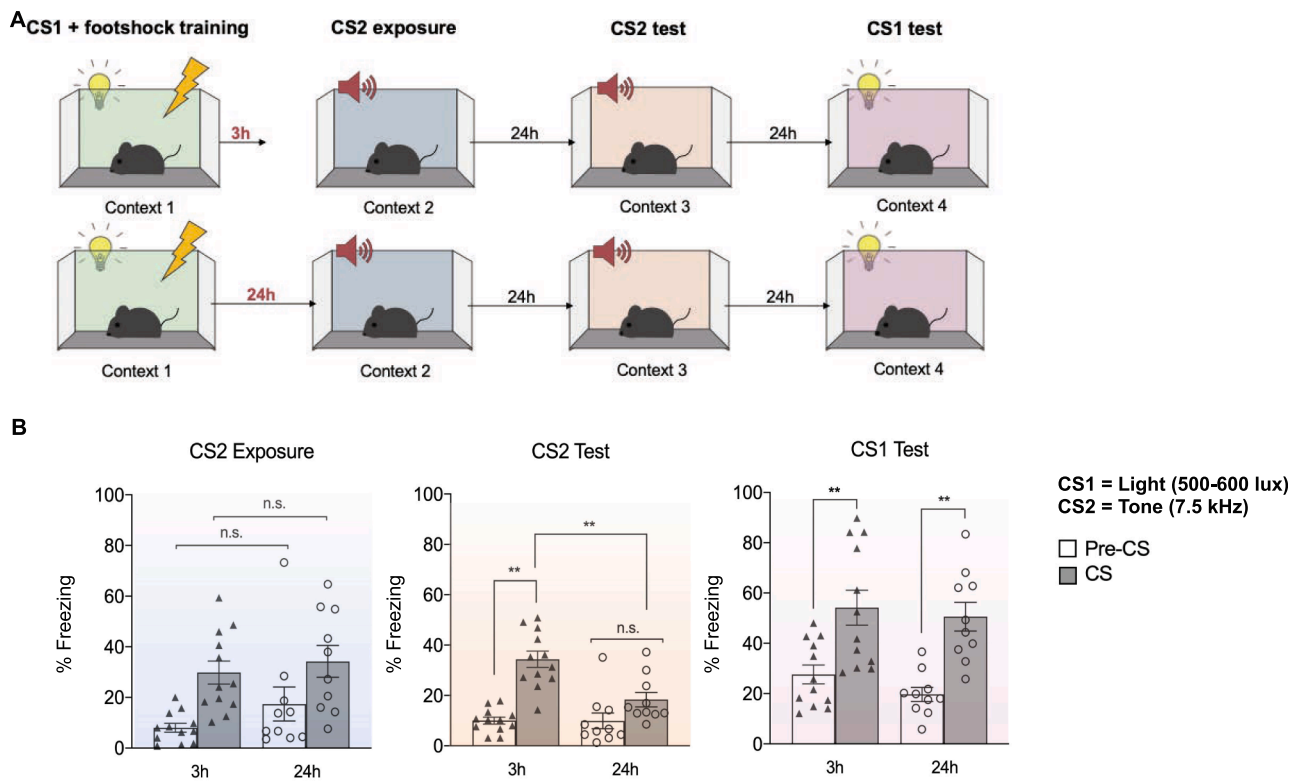
Fig. 3. Behavioral generation of a "false fear memory" in mice using visual and auditory stimuli. (A) 3 h or 24 h after light fear conditioning (CS1 + footshock), mice presented with novel motivationally-neutral CS2 tone without footshock. Freezing assessed upon re-exposure to CS1 (real conditioned fear memory) and CS2 (false memory). (B). During the memory tests, 3 h mice (n = 12) froze more to CS2 than 24 h mice (n = 10). No differences in freezing during initial CS2 exposure, to CS1 in memory test, or pre-CS.

shock pairings (2 min inter-pairing interval) and 30 s later, were re-turned to home-cage.

Exposure to an initially motivationally-neutral conditioned stimulus (CS2). Either 3 or 24 h after cued fear conditioning, mice were placed in a novel context, and 2 min later, a novel motivationally-neutral audi-tory CS (CS2; 7.5 kHz pips, 5 ms rise, 75 dB, 1 min) was presented. Mice were returned to home-cage 30 s later.

Cued fear memory testing. To assess fear memory to the trained sti-mulus (CS1) and the initially motivationally-neutral stimulus (CS2), we measured the percentage time mice spent freezing during CS1 and CS2 presentation. Freezing is an active defensive response defined as ces-sation of movement, except for breathing (Blanchard & Blanchard, 1969; Fanselow & Lester, 1988). Mice were placed in a novel context and 2 min later, CS1 or CS2 was presented (1 min). Only mice with a strong conditioned fear memory (> 25% freezing to CS1) were in-cluded in subsequent analysis (~14% of mice excluded).

2.6. Optogenetic procedures

Assessing neuronal allocation to an engram by optogenetic "priming" of neurons using behavior (Fig. 4). To verify behaviorally that brief opto-genetic activation of a small random number of LA neurons before auditory fear conditioning biases their allocation into the engram supporting this memory, we assessed memory when these same infected neurons were optogenetically inhibited. Specifically, before con-ditioning, we photostimulated mice expressing NpACY in the LA (Fig. 4A) with BL (BL+, 473 nm, 20 Hz, 5 ms pulse width, ~1mW output, 30 s, to activate ChR2 and increase the excitability of infected neurons). Memory was assessed 24 h later by placing mice in a unique context and replaying the tone CS under two conditions (the order of which was counterbalanced); in the absence (RL−) and presence of RL inhibition (RL+, 660 nm, ~7 mW output, 1 min, to activate NpHR3.0

and inhibit neuronal activity) of infected neurons (Fig. 4B). A control group did not receive BL stimulation before training (BL−) but was tested with and without RL. This control group examined the effects of inhibiting a small, non-allocated population of neurons.

Assessing neuronal allocation to an engram by optogenetic "priming" of neurons using immediate early gene immunohistochemistry (Fig. 5). To examine the effects of optogenetically activating a small population of neurons alone or before fear conditioning (Fig. 5) on neuronal activity, we used c-fos immunohistochemistry. Three groups of mice micro-injected with HSV-NpACY were used. One group received 30 s of BL immediately before auditory fear conditioning (as in Fig. 4B, BL + FC). A second group was similarly fear conditioned but did not receive BL (FC) while a third group received BL but were not fear conditioned (BL +). 90 min after training, mice were perfused and c-fos im-munohistochemistry performed, as previously described (A. Park et al., 2020).

Briefly, 8–10 coronal brain sections (50 μm) per mouse, spanning the antero-posterior axis of the LA, were processed and imaged in parallel under identical conditions. Sections were incubated (overnight at 4°C) with rabbit anti-fos polyclonal antibody (1:1000, Synaptic Systems #226 003) and chicken anti-GFP antibody (to amplify NpACY YFP signal) (1:1000, Aves Labs #H1004), followed by incubation (2 h, room temperature) with Alexa568-conjugated goat anti-rabbit and Alexa488-conjugated goat anti-chicken secondary antibodies (1:1000 each, Life Technologies, #A-11011 and #A-110391). Sections were counterstained with DAPI to visualize nuclei. Images were acquired (Zeiss LSM710 confocal microscope) and 3 sections per mouse were analyzed (ImageJ software). The total number of fos+ (active), GFP+ (infected), DAPI + and fos+/GFP+ neurons were counted manually per section by two experimenters unaware of the experimental condi-tions.

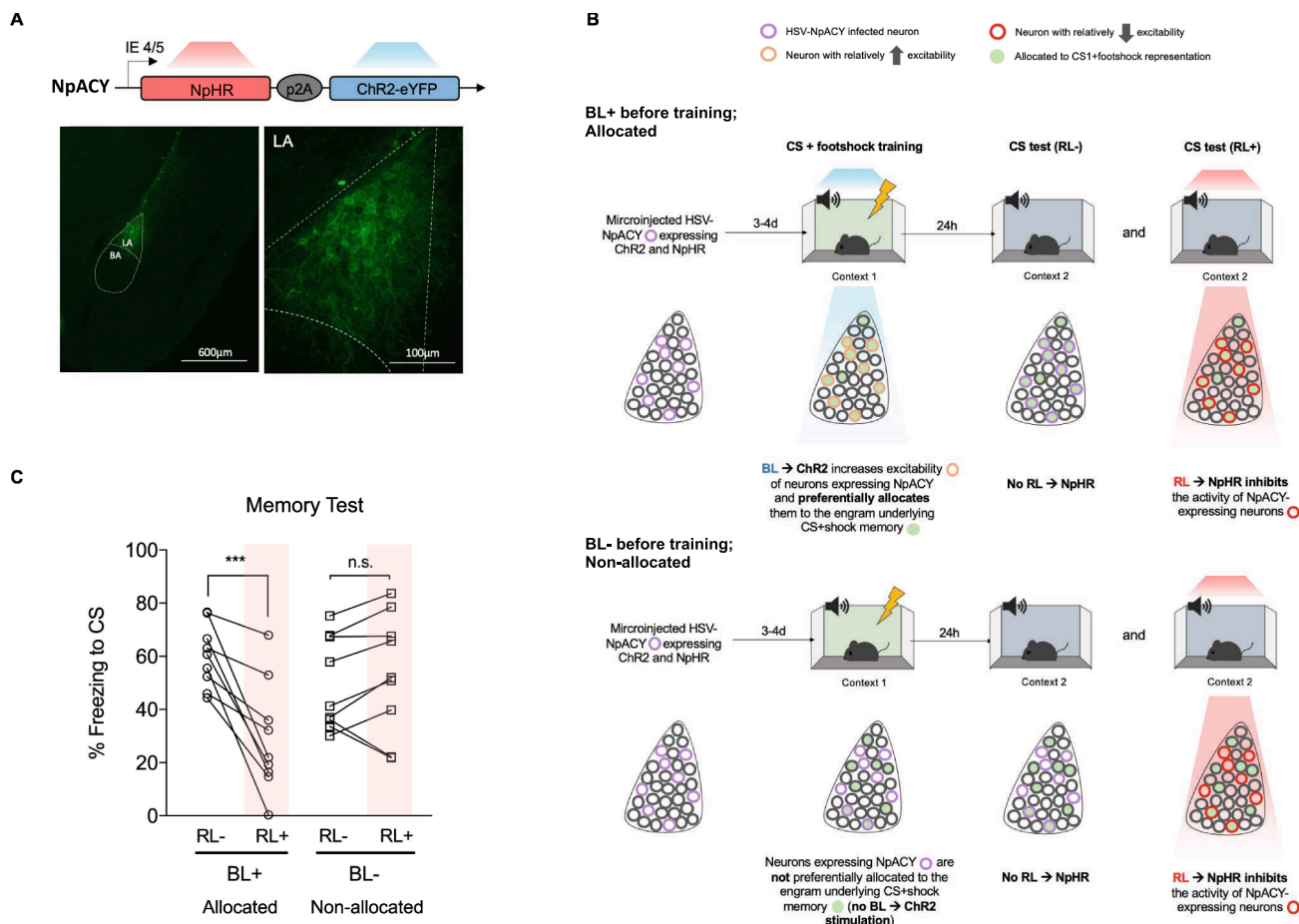*Assessing neuronal allocation to an engram by optogenetic "priming" of*

Fig. 4. Verifying the "allocate-and-manipulate" strategy using behavior. (A) HSV-NpACY construct, expressing both blue light (BL)-responsive excitatory opsin channelrhodopsin [ChR2(H134R)-eYFP] and red light (RL)-responsive inhibitory opsin halorhodopsin (eNpHR3.0), allowed optogenetic activation or inhibition of the same population of infected neurons. Robust expression of NpACY in small population of pyramidal neurons (eYFP) in lateral amygdala (LA) but not basal amygdala (BA). (B) Design of behavioral experiment verifying optogenetic biasing of NpACY-expressing neurons to engram supporting auditory fear memory. Mice expressing HSV-NpACY in random LA neurons (neuron outlined in purple) were fear conditioned (CS + footshock) either immediately after BL photostimulation (to activate ChR2, increase the excitability of these neurons [neuron outlined in orange] and bias their allocation to the underlying engram [green filled neuron], BL+ before training, allocated), or not (BL− before training, non-allocated). Memory was assessed by presenting the tone CS either in the absence (RL−) or presence (RL +) of RL to inhibit the activity of NpACY-expressing neurons [neuron outlined in red]. (C) During memory test, both groups (BL+, n = 9, BL−, n = 9) froze similarly to the CS in RL− condition, but BL+ group froze less when NpACY-expressing neurons were inhibited with RL, verifying that optogenetically "priming" neurons biased their allocation to engram supporting conditioned fear memory. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

neurons using "engram tagging". As an additional means of examining whether optogenetic "priming" of LA neurons before auditory fear conditioning preferentially allocates these neurons to an activity-tagged engram, we used the RAM system (Sørensen et al., 2016) to label with GFP active engram neurons during auditory fear conditioning (Fig. 6). To optogenetically allocate neurons to the GFP-tagged engram (from AAV-RAM-GFP expressing neurons), we also microinjected mice with HSV-ChR2-mCherry. HSV-ChR2-mCherry infects a small number of LA pyramidal neurons while AAV-RAM-GFP infects a much larger number of LA neurons. If optogenetically "priming" neurons biases their allocation to an engram underlying auditory fear conditioning, we would expect a large overlap between optogenetically-primed (mCherry+) in the RAM-GFP-tagged population. In contrast, we would expect to see a relatively smaller overlap between in opsin-expressing mCherry+ neurons if these neurons were not optogenetically primed.

Mice microinjected with AAV-RAM-GFP were maintained on a DOX (40 mg/kg)-containing diet to prevent activity-dependent GFP expression from the RAM system (Fig. 6A). After 17d to allow for AAV expression, mice were microinjected with HSV-ChR2-mCherry while being fed a DOX diet for an additional 2d. 24 h after DOX removal, mice in 4 different treatment groups were trained (Fig. 6A). [1] Mice in the BL-

fear conditioning (BL + FC) group received BL immediately before auditory fear conditioning. [2] Mice in the fear conditioning (FC) alone group were similarly treated did not receive BL. [3] Mice in the BL+ only group received BL but were not fear conditioned. [4] Because not every LA neuron would be infected by the AAV-RAM-GFP vector (and therefore have a potential of being tagged with GFP) in these experiments, we included a control group that was similarly microinjected with HSV-ChR2-mCherry and a control AAV virus expressing GFP in a non-activity-dependent manner (rather than the AAV-RAM-GFP vector). This group allowed the extent of random co-infection of HSV and AAV in LA pyramidal neurons to be assessed, thus serving to mark the "floor" for potential overlap between optogenetically-activated (HSV) and tagged (AAV-RAM-GFP) neurons in the 3 experimental groups.

48 h after training (to allow for activity-dependent expression of GFP from the AAV-RAM-GFP virus) (Sørensen et al., 2016), mice were perfused and brain sections counterstained with DAPI to visualize nuclei. The number of GFP + (active tagged neurons via the AAV-RAM system), mCherry + (neurons expressing ChR2) and total number of DAPI + LA neurons per image were manually counted by two experimenters unaware of experimental conditions.
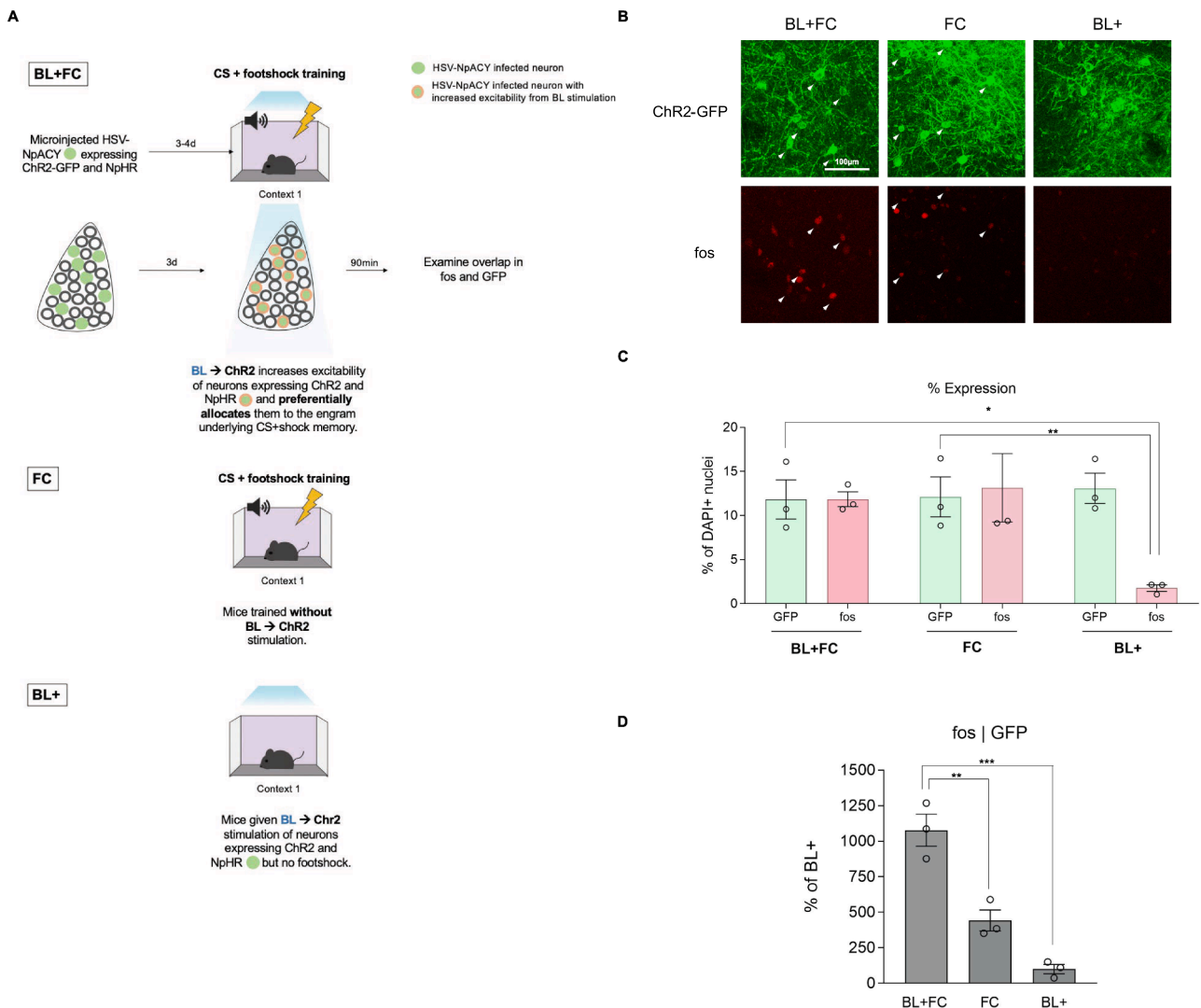
Fig. 5. Verifying the "allocate-and-manipulate" strategy using activity-dependent gene expression. (A) Effects of optogenetically activating neurons alone or before fear conditioning on neuronal activity were examined using fos expression. Three groups expressing HSV-NpACY (green filled neurons) were used (n = 3). [1] BL + FC mice received BL immediately before fear conditioning (FC) to increase the excitability of NpACY-expressing neurons (neuron outlined in orange). [2] FC mice were fear conditioned without BL. [3] BL+ mice received BL but were not fear conditioned. (B) Histology showing co-localization between GFP + (NpACY-expressing) neurons with fos-expressing neurons (red). (C) Similar number of NpACY-infected (GFP +) neurons across groups, but more fos-expressing neurons in BL + FC and FC groups. (D) High overlap of c-fos + and GFP + neurons in BL + FC mice, little overlap in BL + alone mice, indicating BL + FC biased allocation to neurons active during training (proxy of engram) and that BL+ activation of a small population of neurons not sufficient to induce c-fos expression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Assessing the role of excitability-based neuronal allocation to an engram to the formation of a false cued fear memory (Fig. 7). In Fig. 2, we observed that a false memory for CS2 was formed if CS2 was presented 3 h, but not 24 h, after auditory fear conditioning (Event1). We hypothesized that the false memory was formed in the 3 h condition because engram neurons supporting Event1 remained more excitable than their neighbors and that CS2 co-allocated with these neurons. On the other hand, a false memory was not formed if CS2 was presented 24 h post-training because allocated engram neurons for Event1 were no longer more excitable than their neighbors. We tested this hypothesis in two ways.

First, we examined whether inhibiting engram neurons during CS2 presentation 3 h after Event1 disrupted the formation of the false memory. We used BL before CS1 + footshock training to bias the allocation of HSV-NpACY-expressing neurons (Fig. 7A) and inhibited the activity of this same population of neurons with RL during CS2 presentation (Fig. 7A, 3 h-NpACY-RL). To control for photostimulating allocated engram neurons during CS2 exposure, we included a group

that was similarly treated, but received BL (rather than RL) during CS2 exposure (3 h-NpACY-BL). We hypothesized this manipulation would not produce a behavioral effect, as engram neurons would already be more excited than neighboring neurons at this 3 h time point. A second control group expressing HSV-TdTomato received BL before training and RL during CS2 exposure (3 h-TdTomato-RL).

Second, we examined whether optogenetically activating engram neurons during CS2 presentation 24 h after Event1 facilitated the formation of the false memory. Similar to above, we used BL to bias the allocation of HSV-NpACY-expressing neurons to Event1 (Fig. 7A). Then, during CS2 presentation 24 h later, we again used BL to artificially activate these neurons (24 h-NpACY-BL). To control for photostimulating allocated engram neurons during CS2 exposure, we included a group that was similarly treated, but received RL (rather than BL) during CS2 exposure (24 h-NpACY-RL). We hypothesized this manipulation would not impact behavior, as engram neurons would already be less excited than neighboring neurons at this 24 h time point. A second control group expressing HSV-TdTomato received BL before
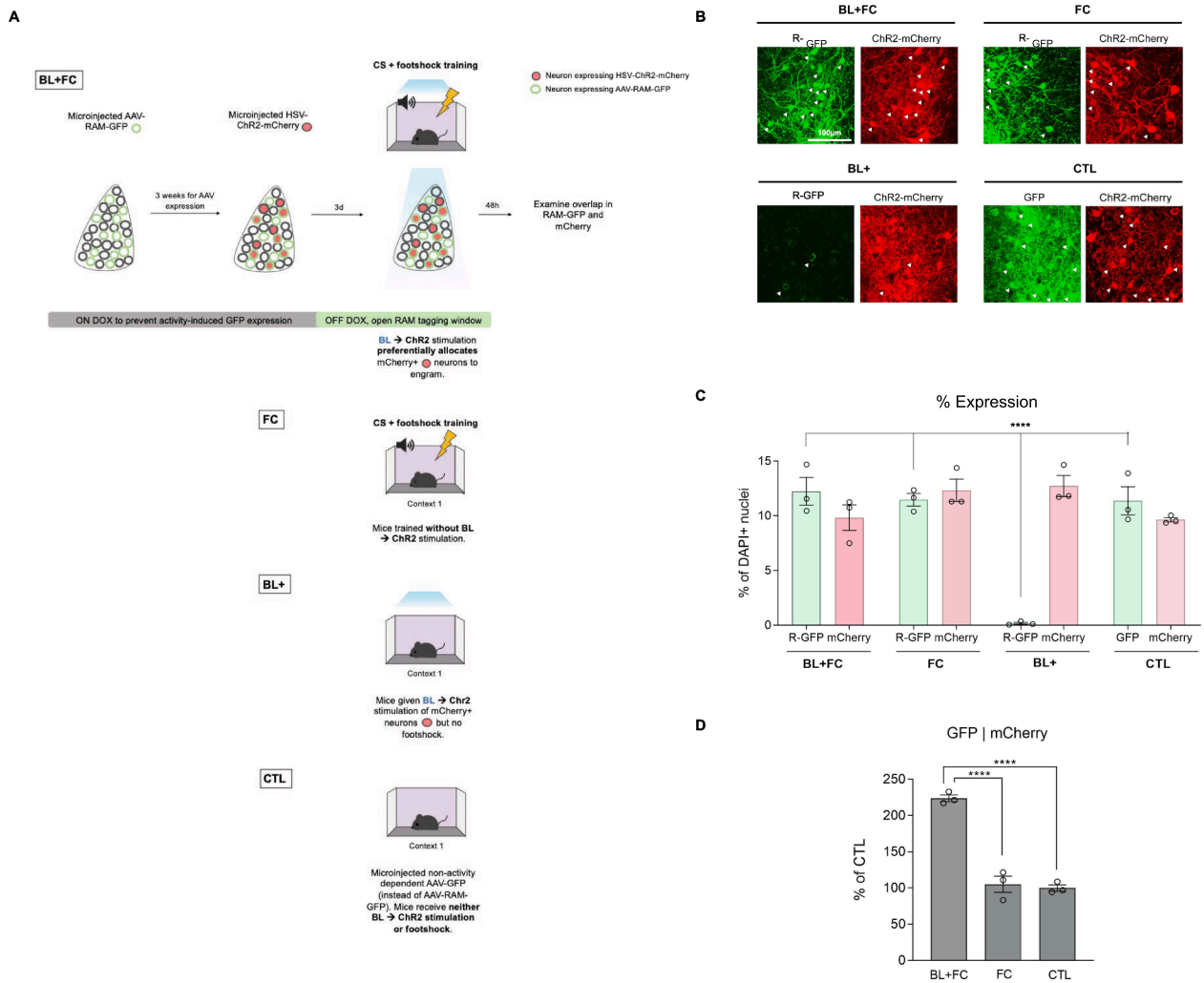
Fig. 6. Verifying the "allocate-and-manipulate" strategy using engram tagging technology. (A) To examine the effects of optogenetically activating a small random number of neurons alone or before fear conditioning on allocation to an engram, engram cells (those active during fear conditioning) were tagged using AAV-RAM-GFP (Sørensen et al., 2016). In the absence of doxycycline (DOX), AAV-RAM-GFP labels with GFP active engram neurons during fear conditioning. 3 groups expressing both AAV-RAM-GFP (green outlined neuron, referred to as R-GFP) and HSV-ChR2-mCherry (red filled neuron) were used (n = 3). [1] BL + FC mice received BL immediately before fear conditioning. [2] FC mice were fear conditioned without BL. [3] BL+ received BL but were not fear conditioned. Control (CTL) mice expressed HSV-ChR2-mCherry and control AAV virus expressing GFP in a non-activity-dependent manner (rather than RAM vector, referred to as GFP). (B) Histology showing overlap of GFP+ and mCherry+ neurons in different experimental groups. Note R-GFP is derived from AAV-RAM-GFP whereas GFP in CTL condition derived from AAV-GFP. (C) Similar number of neurons expressing mCherry across conditions verified similar expression of ChR2-mCherry across groups. High numbers of neurons expressing R-GFP except in mice that were not fear conditioned (BL+) indicated similarly-sized engrams across conditions. (D) Higher overlap (GFP+, mCherry+) in BL + FC mice than in mice that received FC alone, which was not different from the control "floor" condition (CTL). Together, these finding verify this "allocate-and-manipulate" approach to target engram neurons after a training event. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Event1 and CS2 exposure (24 h-TdTomato-BL).

Assessing whether co-allocation mediates a "real" and a false fear memory (Fig. 8). NpACY-expressing neurons were allocated to the engram supporting Event1 (Fig. 8A). 3 h later, mice were exposed to CS2 without optogenetic manipulation (to create a false memory). 24 h later, mice were tested for freezing to CS2 both with and without RL-silencing of NpACY-expressing neurons (CS2 presented for 1 min, 30 s without RL, then 30 s with RL). Mice were tested similarly for CS1 freezing (RL− then RL+) 24 h later. Control mice expressed HSV-NpACY but did not receive BL before Event1 conditioning (such that infected neurons were not experimentally allocated to the engram). These control mice received RL photostimulation during testing sessions identical to that of experimental mice, to examine potential effects of RL inhibition of a small subset of non-experimentally allocated LA neurons.

2.7. Verifying microinjection site and extent of viral infection

Mice were anesthetized deeply with chloral hydrate (1000 mg/kg, ip) or sodium pentobarbital (100 mg/kg, ip) and transcardially perfused with phosphate-buffered saline (PBS, 0.1 M) and 4% paraformaldehyde (PFA). Coronal sections (50 μm) were collected. Rabbit anti-GFP primary antibody (1:1000 dilution; 1891900, Invitrogen, OR) and goat anti-rabbit AlexaFluor 488 secondary antibody (1:500; 1141875, Invitrogen, OR) were used to visualize YFP expression and assess the extent of viral expression. TdTomato fluorescence was examined without amplification. Brain sections were imaged using an epifluorescent microscope. Only mice with robust bilateral expression limited to the LA were included in statistical analyses.
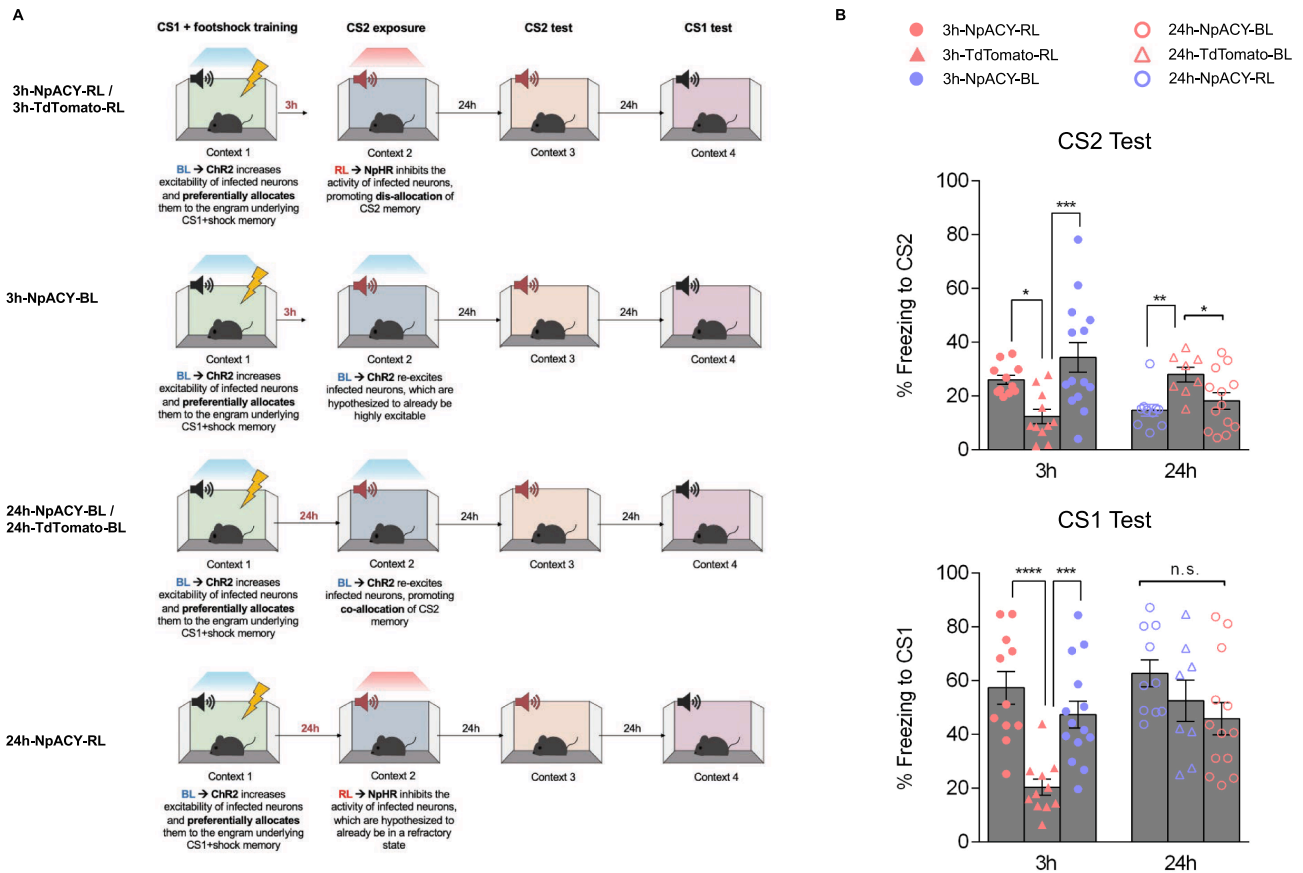
Fig. 7. Using "allocate-and-manipulate" strategy to examine neuronal mechanisms underlying creation of a false memory. (A) Experimental timeline. 3 h condition (top). We hypothesized a false memory was generated if CS2 was presented 3 h post-fear conditioning because engram neurons remain more excitable for several hours. To test this, we first allocated neurons to Event1 engram (BL before CS1 + footshock training) in mice expressing NpACY. During CS2 exposure 3 h later, at a time when NpACY-expressing engram neurons were hypothesized to be more excitable than their neighbors, NpACY-expressing neurons were inhibited (RL) (3 h-NpACY-RL). Control mice were treated similarly, but received BL (not RL) during CS2 exposure (3 h-NpACY-BL) or expressed TdTomato (not NpACY) (3 h-TdTomato-RL). 24 h condition (bottom). NpACY-expressing neurons were allocated to Event1 engram with BL. During CS2 exposure 24 h later, at a time when infected engram neurons are hypothesized to be less excitable than their neighbors, NpACY-expressing neurons were excited (BL) (24 h-NpACY-BL). Control mice received RL (not BL) during CS2 exposure (24 h-NpACY-RL) or expressed TdTomato (not NpACY) (24 h-TdTomato-BL). (B) *CS2 freezing* 3 h Condition. Optogenetically inhibiting, but not exciting, engram neurons, impaired creation of false memory to CS2 when presented 3 h post-fear conditioning (TdTomato RL, n = 11; NpACY-RL, n = 11; NpACY-BL, n = 14). 24 h Condition. Optogenetically activating, but not inhibiting, engram neurons, facilitated creation of a false memory to CS2 when presented 24 h post-fear conditioning (TdTomato-BL, n = 10; NpACY-BL, n = 8; NpACY-RL, n = 13). These findings agree with our hypothesis outlined in Fig. 1B. *CS1 freezing.* All groups froze similarly to the trained CS1 except for 3 h-NpACY-RL condition; inhibiting allocated neurons 3 h post-training disrupted long-term memory formation.

2.8. Statistical analysis

In Figs. 2 and 3, a three-way Analysis of Variance (ANOVA) was used to assess the time spent freezing [between-group factor *Treatment* (3 h vs. 24 h), within-group factors *Sessions* (CS2 Exposure, CS2 Test, CS1 Test) and *Test Phase* (pre-CS vs. CS freezing)]. Significant interactions and main effects were examined using Tukey's HSD post-hoc tests, unless otherwise stated. Comparisons with $p < 0.05$ were considered statistically reliable. For the sake of clarity, we presented baseline (pre-CS) freezing for these figures only. This general pattern of low baseline pre-CS freezing across treatment groups was observed across experiments.

In Fig. 4, a three-way ANOVA with between group factor *BL during training* [BL+ (allocated to CS1) vs. BL− (non-allocated to CS1)] and within-group factor *RL* (RL+ vs. RL−) was used to analyze freezing to CS1 and CS2. In Fig. 5C and 6C, two-way ANOVAs with between group factor *Treatment* (BL + FC, FC, BL+) and within-group factor *Labeling* (GFP vs. fos or R-GFP vs. mCherry) was used, while in Fig. 5D and Fig. 6D, one-way ANOVAs were used. In Fig. 7, one-way ANOVAs were used to analyze freezing in the 3 h and 24 h groups in both CS2 and CS1 tests and Fisher's LSD tests were used for post-hoc analysis. In Fig. 8, a three-way ANOVA with between group factor *BL during training* [BL+

(allocated to CS1) vs. BL− (non-allocated to CS1), and within-group factors *RL at test* (RL+ vs. RL−) and *Sessions* (CS2 Test, CS1 Test)] was used.

3. Results

3.1. Mice show fear responses to a neutral stimulus presented shortly (3 h), but not a longer (24 h) time, after auditory fear conditioning

We first trained all mice on auditory cued fear conditioning during which a tone (CS1) was paired with a footshock. Either 3 h or 24 h later, mice were placed in a new context and a novel, motivationally-neutral tone (CS2) was presented without footshock (Fig. 2A). 24 h later, mice were placed a unique context and CS2 was replayed. During the memory test, mice exposed to CS2 3 h after fear conditioning showed higher CS2 freezing than mice exposed to CS2 24 h after conditioning, even though in both groups, CS2 was never paired with footshock. In contrast, both groups showed similar freezing when initially exposed to CS2 (either 3 h or 24 h after CS1 + footshock training), to CS1 during a memory test, and before CSs presentations (pre-CS freezing) (Fig. 2B). Results of a three-way ANOVA support this interpretation [*Treatment* (3 h vs. 24 h) × *Session* (CS2 Exposure, CS2 Test, CS1 Test) × *Test*
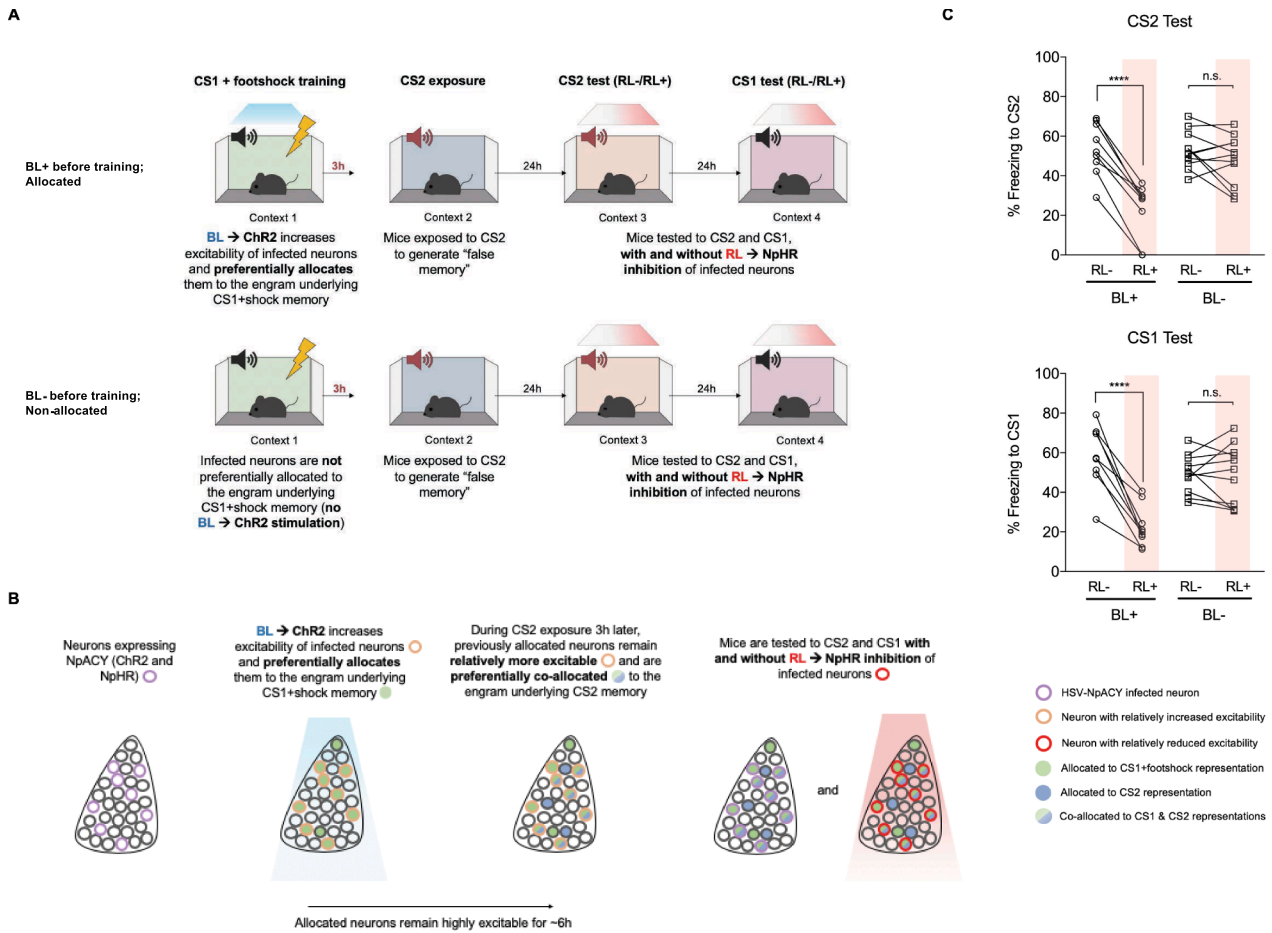
Fig. 8. False memory formation depends on excitability-mediated neuronal co-allocation. (A) Experimental timeline. (Top) BL + before training (Allocated). NpACY-expressing neurons were allocated to CS1 + footshock engram via BL. Mice exposed to CS2 3 h later without photostimulation. Memory (CS1 and CS2) was assessed when NpACY-expressing neurons were (and were not) inhibited (RL + vs. RL−). (Bottom) BL− before training (Non-allocated). Control mice treated similarly, but did not receive BL before CS1 + footshock training, such that infected neurons were not preferentially allocated to the engram. (B) Schematic depicting NpACY-expressing neurons (neurons outlined with purple) being preferentially allocated to CS1 + footshock engram (green filled neurons) via BL stimulation (to increase excitability, orange outline). 3 h later, during CS2 exposure, allocated engram neurons are thought to remain more excitable than their neighbors (orange outlined neurons) and co-allocate CS2 representation to overlapping neurons (green and blue neurons). By virtue of co-allocation, decreasing activity of NpACY-expressing neurons via RL (red outlined neurons) would decrease freezing to both CS1 and CS2 during memory test. (C) During CS1 and CS2 test, mice in experimental allocation group (n = 11) froze less during RL +, than RL−, suggesting NpACY-expressing neurons supported both real and false fear memories. Non-allocated mice (n = 9) showed equivalent freezing in RL +/- conditions, indicating inhibition of a small number of LA neurons failed to impact freezing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Phase (pre-CS vs. CS freezing) ANOVA revealed significant interactions of *Treatment* × *Session* × *Test Phase* ($F_{2, 46} = 8.40, p < 0.001$), *Session* × *Treatment* ($F_{2, 46} = 4.01, p < 0.05$), *Session* × *Test Phase* ($F_{2, 46} = 15.58, p < 0.001$), as well as significant main effects of *Session* ($F_{2, 46} = 16.71, p < 0.001$) and *Test Phase* ($F_{1, 23} = 221.5, p < 0.001$); post-hoc comparisons showed 3 h group froze > 24 h group during CS2 presentation at CS2 testing, but that groups did not differ during CS2 exposure, CS1 test or during pre-CS]. That both groups froze similarly when initially exposed to CS2 (either 3 or 24 h after cued fear conditioning) indicates that 3 h mice were not simply more sensitized to any new stimulus.

Next, we directly compared freezing levels between mice exposed to CS2 either 3 or 24 h post-training to mice that were not exposed to CS2 after training (no exposure, NE) and first heard CS2 during the test session (Fig. 2C). All groups froze similarly to CS1 during the test ($F_{2, 28} = 1.29, p > 0.05$), indicating that memory for the original training event was not impacted by the presence or timing of subsequent exposure to CS2. Importantly, mice in the 24 h group froze at similar levels to CS2 during the test than the group of mice hearing this tone for the first time ($F_{2, 28} = 10.88, p < 0.001$; post-hoc comparisons showed no difference between NE and 24 h group, but higher freezing in 3 h

group). These results suggest exposure to CS 24 h after conditioning is roughly equivalent to a novel CS at test, at least in terms of freezing behavior.

In these experiments (Fig. 2), both CSs (CS1 that was paired with footshock and CS2 that was not paired with footshock) were auditory. To examine whether the observed false memory effect could be attributed to tone generalization, we changed CS1 to a light cue (Fig. 3). During training, all mice received two light (CS1) + footshock pairings. Either 3 or 24 h later, a novel, motivationally-neutral tone (CS2) was presented (Fig. 3A). Similar to experiment using two auditory stimuli (CS1, CS2), mice showed increased freezing to the tone CS2 during the test session – despite it being a different sensory modality to the trained CS1-light – only if CS2 was presented 3 h, but not 24 h, after cued fear conditioning [Fig. 3B; three-way ANOVA revealed significant *Session* × *Treatment* × *Test Phase* ($F_{2, 38} = 3.53, p < 0.05$) and *Session* × *Test Phase* ($F_{2, 38} = 4.00, p < 0.05$) interactions, and significant main effects of *Session* ($F_{2, 38} = 18.79, p < 0.001$) and *Test Phase* ($F_{1, 19} = 131.50, p < 0.001$); post-hoc comparisons showed 3 h mice froze more than 24 h mice to CS2 at test, but no difference between freezing during CS2 exposure, or CS1 at test]. Again, both groups showed similar levels of freezing when initially exposed to CS2, during the pre-CS

period at testing and when tested to the original stimulus (CS1; which was paired with footshock).

These findings are consistent with our previous results in which memory for a new fear conditioning event (Event2, CS2 + footshock) depended on the timing and content of a previous event (Event1). That is, mice showed higher freezing to CS2 if Event1 occurred 1.5–6 h (but not longer) before Event2, only if Event1 consisted of CS1 + footshock training (not simply CS1 exposure or an immediate footshock) (Rashid et al., 2016). Interestingly, mice receiving an immediate footshock 6 h before Event2 did not show enhanced CS2 freezing levels indicating the increase in freezing depended on learning, rather than simply arousal or sensitization (Rashid et al., 2016).

3.2. Examining an "allocate-and-manipulate" strategy to investigate the creation of a false memory

We hypothesized that excitability-based neuronal allocation to an engram underlies the observed behaviorally-induced false memory (see Fig. 1). If Event1 engram neurons remained relatively more excited than their neighbors for several hours, then presentation of a novel motivationally-neutral CS2 during this period may also engage these engram neurons, thereby creating a false fear memory to CS2. One way to investigate the contribution of excitability-based neuronal allocation to the observed false memory, is to optogenetically inhibit Event1 engram neurons during CS2 exposure 3 h post-Event1.

Because a tag-and-manipulate strategy is not ideal for these studies (see Methods), we used an "allocate-and-manipulate" strategy. Increasing the excitability of a small population of LA neurons, using a variety of methods, biases their allocation to an engram (Park et al., 2020; Park et al., 2016; Rashid et al., 2016; Yiu et al., 2014; Zhou et al., 2009). Here, we used three strategies to verify optogenetic allocation of neurons to an engram supporting a conditioned fear memory.

First, to behaviorally verify it is possible to optogenetically bias the allocation of infected neurons into the engram supporting a conditioned fear memory (Fig. 4B), we first used BL to artificially activate NpACY-expressing neurons in mice before training, and tested memory when the same infected neurons were optogenetically inhibited. A control group was similarly treated, but did not receive BL (BL−) before auditory fear conditioning, allowing us to examine the effects of silencing a small, random (but not experimentally allocated) population of neurons on memory.

Both groups (BL+ vs. BL−) showed robust freezing when tested in the RL− condition (with no optogenetic inhibition of infected neurons) (Fig. 4C). However, only mice that were trained immediately after BL stimulation showed a decrease in freezing when NpACY-expressing neurons were inhibited by RL. Importantly, inhibiting a random population of neurons (BL− condition) did not affect freezing. This interpretation is supported by the results of a two-way ANOVA [significant interaction between $BL\ at\ Training \times RL\ at\ Test$ ($F_{1,\ 16} = 22.44$, $p < 0.001$), as well as main effects of $RL\ at\ Test$ ($F_{1,\ 16} = 13.46$, $p < 0.05$); post-hoc comparisons showed lower freezing during RL+ inhibition only in the BL+ group]. These results indicate neurons optogenetically activated (with BL) before training preferentially become allocated to the engram as inhibition of these neurons (and not a similar number of random neurons) impaired subsequent memory expression.

Second, to examine the effects of briefly optogenetically activating a small population of LA neurons alone or before fear conditioning (Fig. 5), we used c-fos immunohistochemistry as a marker of neuronal activity. Three groups of mice expressing NpACY were used. BL + FC mice received BL immediately before auditory fear conditioning (as in Fig. 4). FC mice were similarly fear conditioned but did not receive BL before training. BL+ mice received BL-photostimulation but were not fear conditioned. All groups showed similar levels of NpACY expression (GFP+ neurons), indicating similar infection levels (Fig. 5C). However, both fear conditioned groups (BL + FC, FC) showed more c-fos+ neurons than BL+ group [significant $Treatment$ (BL + FC, FC, BL

+) $\times$ $Labeling$ (GFP vs. fos) interaction ($F_{2,\ 6} = 17.4$, $p < 0.01$), as well as a significant main effect of $Labeling$ ($F_{1,\ 6} = 13.0$, $p < 0.05$); post-hoc comparisons showed no difference in GFP levels across groups, but higher c-fos+ neurons in both fear conditioned groups (BL + FC, FC) compared to BL+ group]. These results suggest that BL+ alone was not sufficient to induce c-fos expression. Moreover, consistent with the interpretation of optogenetic priming of allocation, we observed a high overlap of c-fos+ and GFP+ neurons in BL + FC mice [Fig. 5D; ($F_{2,\ 6} = 38.0$, $p < 0.001$); post-hoc comparisons showed a greater overlap in the BL + FC group compared to other groups, which did not differ].

Finally, to further examine whether optogenetic "priming" of LA neurons before auditory fear conditioning preferentially allocates these neurons to an engram supporting this memory, we used the RAM "engram labelling" system in an AAV vector (Fig. 6). Neurons active at the time of auditory fear conditioning (putative engram neurons) were tagged with GFP. To allocate neurons, we used HSV-ChR2-mCherry and examined the overlap of mCherry+ (infected) neurons in GFP+ (RAM tagged) neurons in three groups. Similar to above, BL + FC mice received BL immediately before auditory fear conditioning. FC mice were fear conditioned and BL+ mice received photostimulation only. Finally, because we examined the overlap of potentially different populations of neurons infected by two viral vectors (HSV, AAV), we included an additional control (CTRL) group that allowed us to assay the overlap between HSV-ChR2-mCherry+ neurons and neurons infected with an AAV expressing GFP in a non-activity-dependent way (AAV-GFP) (to mark the "floor" of any potential overlaps between AAV-infected and HSV-infected neurons in the experimental groups).

All groups showed a similar number of mCherry+ HSV-infected neurons and tagged GFP+ neurons except mice that were not fear conditioned (BL+) [Fig. 6C; $Treatment$ (BL + FC, FC, BL+, CTL) $\times$ $Labeling$ (R-GFP vs. mCherry) ANOVA showed significant $Treatment \times Labeling$ ($F_{3,\ 8} = 24.0$, $p < 0.001$) interaction, as well as significant main effects of $Treatment$ ($F_{3,\ 8} = 16.0$, $p < 0.01$) and $Labeling$ ($F_{1,\ 8} = 10.6$, $p < 0.05$); post-hoc comparisons showed no difference in mCherry levels across groups, but fewer numbers of GFP+ neurons in BL+ group only]. Moreover, the overlap between engram tagged (GFP+) and HSV-infected (mCherry+) was greater in BL + FC than in FC alone mice, the latter of which was not different from the control "floor" condition [Fig. 6D; $F_{2,\ 6} = 87.0$, $p < 0.0001$; post-hoc comparisons showed higher overlap in the BL + FC group compared to both FC and CTL groups, which did not differ]. These findings, along with the behavioral and c-fos results, support the conclusion that the present optogenetic allocation strategy allows us to target engram neurons. Next, we used this strategy to examine the effects on the creation of a false memory of manipulating the activity of engram neurons after training.

3.3. Post-training interference with the heightened excitability of engram neurons disrupts false memory formation

To investigate whether excitability-based neuronal allocation underlies the formation of the types of false memories we observed in Figs. 2 and 3, we first biased the allocation of NpACY-expressing neurons to the engram (BL+ before auditory fear conditioning). Then, during exposure to CS2 (without footshock) 3 h later, the activity of the same infected neurons was inhibited with RL (3 h-NpACY-RL) (Fig. 7A). A control group expressing TdTomato was treated similarly (BL before fear conditioning, RL during CS2 exposure, 3 h-TdTomato-RL).

Mice in the 3 h control group (3 h-TdTomato-RL) showed ~30% freezing to CS2 during the memory test (Fig. 7B), similar to Fig. 2, suggesting a false memory. However, inhibiting engram neurons allocated to Event1 prevented the formation of a false memory; mice in the 3 h-NpACY-RL group showed lower CS2 freezing in the memory test than control mice (Fig. 7B). Importantly, artificially reactivating allocated neurons during exposure to CS2 3 h after fear conditioning (3 h-NpACY-BL) did not occlude or facilitate the creation of a false memory;

mice in this condition showed similar CS2 freezing levels as control mice expressing TdTomato alone [Fig. 7B; 3 h group CS2 test; $F_{2, 33}$ = 7.54, p < 0.05; post-hoc comparisons showed 3 h-NpACY-RL froze less than 3 h-TdTomato-RL controls or 3 h-NpACY-BL, which did not differ]. These results are consistent with the idea that, in control mice, the initially motivationally-neutral CS2 acquired aversive properties via the elevated excitability of Event1 fear engram neurons during CS2 exposure. Inhibiting Event1 engram neurons during CS2 exposure resulted in no false memory, perhaps mimicking the endogenous condition in which CS2 exposure occurred 24 h after cued fear conditioning, at a time when the engram neurons from Event1 would no longer be hyper-excitable. Artificially reactivating engram neurons 3 h after auditory fear conditioning (CS2 presentation) does not further enhance memory integration and the formation of a false memory (as these neurons may already be more excitable, 3 h-NpACY-BL group).

3.4. Artificially exciting engram neurons facilitates creation of a false memory

Our behavioral studies indicate that a false memory is not created if a novel, motivationally-neutral CS2 is presented 24 h after fear conditioning. We next asked if it is possible to artificially co-allocate normally distinct CS1 + footshock and CS2 representations to create a false fear memory to CS2 using optogenetics. We attempted to experimentally mimic the natural neural state when CS2 is presented 3 h after training (when engram neurons representing CS1 + footshock memory are more excitable than their neighbours). As before, we first biased the allocation of infected neurons to Event1 (CS1 + footshock) by photo-stimulating infected neurons with BL before training. Then, 24 h later, we optogenetically reactivated these neurons before CS2 exposure (without footshock) with BL (Fig. 7A; 24 h-NpACY-BL). Mice with control virus expressing fluorophore alone were treated similarly (24 h-TdTomato-BL).

As expected, control mice (24 h-TdTomato-BL) showed < 20% CS2 freezing during the CS2 test, showing no false memory (Fig. 7B). In contrast, experimental mice in which neurons allocated to Event1 were artificially reactivated before CS2 exposure (24 h-NpACY-BL) showed increased CS2 freezing during the subsequent test, suggesting artificially co-allocating neurons to overlapping engrams supported the creation of a false memory. Importantly, artificially inhibiting allocated neurons during CS2 exposure 24 h after fear conditioning (24 h-NpACY-RL) (at a time we hypothesized these neurons would already be endogenously inhibited) did not impact the low test CS2 freezing (Fig. 7B; 24 h group CS2 test; $F_{2, 28}$ = 5.12, p < 0.05; post-hoc comparisons showed 24 h-NpACY-BL group froze more than 24 h-TdTomato-BL controls or 24 h-NpACY-RL, which did not differ). These data are consistent with the interpretation that a false memory is not created when mice are exposed to CS2 24 h after fear conditioning due to engram dis-allocation via decreased excitability of neurons in the original conditioned fear engram. Lastly, all 24 h groups, regardless of treatment, showed similar CS1 freezing during the memory test (Fig. 7B, 24 h group CS1 test; $F_{2, 28}$ = 2.02, p > 0.05). However, mice in which allocated neurons were inhibited 3 h after conditioning showed lower CS1 freezing than other 3 h groups (3 h group CS1 test; $F_{2, 33}$ = 13.97, p < 0.001; post-hoc analyses showed that the 3 h-NpACY-RL froze < 3 h-TdTomato-RL and 3 h-NpACY-BL controls), similar to previous observations (S.Park et al., 2016).

3.5. Creation of a false memory is endogenously mediated by neuronal co-allocation

Finally, we next asked whether the "real" conditioned fear memory and the false memory we observed were mediated by overlapping populations of neurons. To assess this idea, we tested both freezing to CS1 (the "real conditioned fear memory") and CS2 (the false conditioned

fear memory) when CS1 engram neurons were inhibited with RL. We trained mice expressing NpACY as above (first biasing allocation of NpACY-expressing neurons to the engram underlying the CS1 + footshock memory with BL) and then presented the CS2 (without photostimulation) 3 h later (Fig. 8A; BL+). Control mice were treated similarly, except that NpACY-infected neurons were not biased for allocation to the engram for Event 1 (Fig. 8A; no BL before CS1 + footshock fear conditioning, BL−).

In the non-allocated control group (BL−), mice froze robustly to both CS1 and CS2 regardless of RL photostimulation, indicating that inhibiting a small, random population of neurons did not impact memory for a trained CS or a false memory (Fig. 8B). In contrast, mice that received BL before conditioning showed normal freezing when tested to both CS1 and CS2 in the absence of RL inhibition, but decreased freezing during RL inhibition of NpACY-expressing neurons (Fig. 8B; three-way ANOVA [*BL at Training* (BL+ vs. BL−), *RL at Test* (RL+ vs. RL−) and *CS* (CS1 vs. CS2 testing) revealed a significant interaction of *BL at Training* × *RL at Test* ($F_{1, 18}$ = 67.40, p < 0.001), as well as significant main effects of *BL at Training* ($F_{1, 18}$ = 7.04, p < 0.05) and *RL at Test* ($F_{1, 18}$ = 93.61, p < 0.0001). Post-hoc comparisons showed no difference between RL+ and RL− freezing in the BL− group when tested to CS1 or CS2, but that RL decreased both CS1 and CS2 freezing in the BL+ group]. Together, these results indicate that an overlapping population of neurons played key roles in the engram supporting the actual fear memory and the false memory, as inhibition of the same population impaired freezing to both CS1 and CS2 (Fig. 8C). Finally, these results suggest that the false memory was due to integration of the motivationally-neutral CS2 with the CS1 + footshock representation via excitability-dependent co-allocation.

4. Discussion

Mistaken eyewitness testimony (Smalarz & Wells, 2015), inaccurate or implanted childhood memories (Berkowitz, Laney, Morris, Garry, & Loftus, 2008; Hyman, Husband, & Billings, 1995; Loftus & Pickrell, 1995), and everyday lapses in our ability to recollect the past reveal the fallibility of memory. However, some memory distortions may reflect (be a "bug" of) normal adaptive memory processes. Here, we investigated whether the adaptive process of memory linking can be hijacked to create an entirely false memory in mice using only behavioral manipulations [for conceptually similar work with humans, see (Carpenter & Schacter, 2017)].

Previously, we and others showed that two similar events that occur in close temporal proximity can be linked by virtue of co-allocation of neurons to overlapping engrams, an effect dependent on neuronal excitability (Cai et al., 2016; Rashid et al., 2016). Neurons relatively more excitable at the time of Event1 are allocated to the engram supporting Event1 memory. Moreover, these allocated engram neurons remain more excitable even after Event1. Should a second, related event occur during this time of heightened engram excitability, Event1 engram neurons also allocated to the engram supporting Event2, and by virtue of this co-allocation, memories for Event1 and Event2 become linked.

Previous studies from several types of experiments supported the interpretation of memory linking via co-allocation. First, Event1 and Event2 LA engrams (labeled via *arc*, *homer1a* mRNA) showed greater overlap if the time between these events was relatively short (6 h), rather than long (24 h) (Rashid et al., 2016). Second, extinction of Event2 memory resulted in functional extinction of Event1 memory in the 6 h inter-trial interval, but not 24 h inter-trial interval group (Rashid et al., 2016). Third, CS-induced retrieval of a previously trained CS + footshock memory could become functionally linked to a new learning event (CS2 + footshock) if retrieval was induced 6 h, but not 24 h, before Event2, indicating that functional linking can occur even in the absence of a recent footshock. Although these experiments were done with discrete (typically auditory) cue fear conditioning, a similar

overall pattern of results was reported using contextual tasks (Cai et al., 2016). Here, we took advantage of insights gained from previous memory-linking studies to successfully create a false discrete cued fear memory in mice using purely behavioral procedures.

In the present studies, mice froze to an initially motivationally-neutral tone, only if this tone was presented shortly after cued Pavlovian fear conditioning (a false memory). Importantly, the same tone presented 24 h after fear conditioning did not elicit freezing, indicating the key importance of close temporal proximity. We hypothesized that creation of the false fear memory was due to excitability-dependent co-allocation mechanisms. We found that decreasing the excitability of neurons allocated to Event1 engram during the presentation of the motivationally-neutral CS shortly after fear conditioning prevented false memory formation. Moreover, artificially re-activating Event1 engram neurons during presentation of the motivationally-neutral stimulus 24 h after fear conditioning facilitated the formation of a false memory. Finally, both the original memory and the false memory were supported by overlapping populations of neurons. Together, these findings suggest that this type of false memory derives from excitability-dependent processes that normally serve to link or integrate memories of events occurring in close temporal proximity [for further discussion of neural aspects of memory integration, see (Schlichting & Frankland, 2017; Schlichting & Preston, 2015)].

Previous studies showed it is possible to create false memories in rodents using other behavioral techniques. For example, it has been known for nearly a century (Pavlov, 1927; Watson & Rayner, 1920) that rodents generalize the properties of learned stimuli, and, furthermore, that generalization tends to increase at time points distal to training (Perkins & Weyant, 1958). For instance, rodents generalize contextual fear responses over time, showing higher freezing in a neutral non-shocked context at time points many days, but not 1 day, after contextual fear conditioning (Biedenkapp & Rudy, 2007; Ruediger et al., 2011; Wiltgen & Silva, 2007). Moreover, Silva, Cai and colleagues (Cai et al., 2016) showed that a false contextual memory could be created by exposing mice to distinct contexts in close temporal proximity. In contrast, the present results used behavioral techniques to show that a false fear memory to a discrete cue (a tone) can be created and expressed shortly after training. We then went on to investigate the neural basis for this false memory.

To probe the neural basis of a behaviorally-induced false memory, we used optogenetics to either artificially prevent or create a false memory by mimicking endogenous excitability states of engram neurons (inhibiting the engram at 3 h post-training to mimic the naturally-occurring state of 24 h post-training, re-exciting the engram at 24 h post-training to mimic the endogenous 3 h state). Previous studies showed that it was possible to create false memories using optogenetics in other ways. For example, Ramirez and colleagues (Ramirez et al., 2013) first tagged dentate gyrus neurons representing context A with ChR2. Mice were then fear conditioned to context B while the representation of context A was reactivated optogenetically. When tested, mice froze to context A, even though shock was never presented in this context – a false memory. Moreover, a false memory was created by repeatedly co-presenting the CSs from two independent aversively-motivated tasks (fear conditioning and conditioned taste aversion) (Yokose et al., 2017). Memory for the two tasks became integrated such that a CS used in the conditioned taste aversion training now elicited freezing (even though this CS had never been paired with a shock). Finally, a false memory was recently implanted in mice via optogenetic stimulation without the mice ever experiencing either the actual CS or US (Vetere et al., 2019). Optogenetic stimulation of a genetically-specific olfactory glomerulus (the putative CS) was paired with optogenetic stimulation of aversive neural pathways (the putative USs) (Vetere et al., 2019). Even though the mice had never experienced either the odor associated with this olfactory glomerulus or a footshock, mice avoided the scent when later tested, showing evidence of a false implanted memory.

While these past studies generated false memories using various methods (delayed training-to-testing intervals, generalized fear to a context, optogenetic activation of tagged neural circuits, etc.), the present study generated a false memory for a discrete cue using only behavioral manipulations. By showing that false memories can be generated through the hijacking of the adaptive processes of memory linkage and excitability-mediated co-allocation, these findings shed light on memory processes and how they may go awry.

CRediT authorship contribution statement

Jocelyn M.H. Lau: Methodology, Investigation, Visualization, Writing - review & editing. **Asim J. Rashid:** Resources, Methodology, Investigation, Validation. **Alexander D. Jacob:** Formal analysis, Investigation. **Paul W. Frankland:** Supervision, Conceptualization. **Daniel L. Schacter:** Conceptualization, Writing - review & editing. **Sheena A. Josselyn:** Writing - original draft, Conceptualization, Supervision.

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Disclosures

References

Barrot, M., Olivier, J. D., Perrotti, L. I., DiLeone, R. J., Berton, O., Eisch, A. J., ... Nestler, E. J. (2002). CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proceedings of the National Academy of Sciences of the United States of America, 99*(17), 11435–11440.

Berkowitz, S. R., Laney, C., Morris, E. K., Garry, M., & Loftus, E. F. (2008). Pluto behaving badly: False beliefs and their consequences. *American Journal of Psychology, 121*(4), 643–660.

Biedenkapp, J. C., & Rudy, J. W. (2007). Context preexposure prevents forgetting of a contextual fear memory: Implication for regional changes in brain activation patterns associated with recent and remote memory tests. *Learning & Memory, 14*(3), 200–203. https://doi.org/10.1101/lm.499407.

Blanchard, R. J., & Blanchard, D. C. (1969). Crouching as an index of fear. *Journal of Comparative and Physiological Psychology, 67*(3), 370–375.

Brainerd, C. J., & Reyna, V. F. (2005). *The science of false memory.* New York, NY, US: Oxford University Press.

Bremner, J. D., Shobe, K. K., & Kihlstrom, J. F. (2000). False memories in women with self-reported childhood sexual abuse: An empirical study. *Psychological Science, 11*(4), 333–337. https://doi.org/10.1111/1467-9280.00266.

Cai, D. J., Aharoni, D., Shuman, T., Shobe, J., Biane, J., Song, W., ... Silva, A. J. (2016). A shared neural ensemble links distinct contextual memories encoded close in time. *Nature, 534*(7605), 115–118. https://doi.org/10.1038/nature17955.

Carlezon, W. A., Jr., Nestler, E. J., & Neve, R. L. (2000). Herpes simplex virus-mediated gene transfer as a tool for neuropsychiatric research. *Critical Reviews in Neurobiology, 14*(1), 47–67.

Carlezon, W. A., Jr., & Neve, R. L. (2003). Viral-mediated gene transfer to study the behavioral correlates of CREB function in the nucleus accumbens of rats. *Methods in Molecular Medicine, 79*, 331–350.

Carpenter, A. C., & Schacter, D. L. (2017). Flexible retrieval: When true inferences produce false memories. *Journal of Experimental Psychology. Learning, Memory, and Cognition, 43*(3), 335–349. https://doi.org/10.1037/xlm0000340.

Davis, M. (1992). The role of the Amygdala in fear and anxiety. *Annual Review of Neuroscience, 15*(1), 353–375. https://doi.org/10.1146/annurev.ne.15.030192.002033.

Denny, C. A., Kheirbek, M. A., Alba, E. L., Tanaka, K. F., Brachman, R. A., Laughman, K. B., ... Hen, R. (2014). Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. *Neuron, 83*(1), 189–201. https://doi.org/10.1016/j.neuron.2014.05.018.

Fanselow, M. S., & Lester, L. S. (1988). A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. In R. C. Bolles, & D. Beecher (Eds.). *Evolution and learning* (pp.

185–212). Hillsdale, NJ, US: Lawrence Erlbaum Associates, Inc.

Frankland, P. W., Josselyn, S. A., & Köhler, S. (2019). The neurobiological foundation of memory retrieval. *Nature Neuroscience, 22*(10), 1576–1585. https://doi.org/10.1038/s41593-019-0493-1.

Gallo, D. A. (2006). *Associative illusions of memory: False memory research in DRM and related tasks.* New York, NY, US: Psychology Press.

Garner, A. R., Rowland, D. C., Hwang, S. Y., Baumgaertel, K., Roth, B. L., Kentros, C., & Mayford, M. (2012). Generation of a synthetic memory trace. *Science, 335*(6075), 1513–1516. https://doi.org/10.1126/science.1214985.

Gouty-Colomer, L. A., Hosseini, B., Marcelo, I. M., Schreiber, J., Slump, D. E., Yamaguchi, S., ... Kushner, S. A. (2015). Arc expression identifies the lateral amygdala fear memory trace. *Molecular Psychiatry.* https://doi.org/10.1038/mp.2015.18.

Guenthner, C. J., Miyamichi, K., Yang, H. H., Heller, H. C., & Luo, L. (2013). Permanent genetic access to transiently active neurons via TRAP: Targeted recombination in active populations. *Neuron, 78*(5), 773–784. https://doi.org/10.1016/j.neuron.2013.03.025.

Han, J. H., Kushner, S. A., Yiu, A. P., Cole, C. J., Matynia, A., Brown, R. A., ... Josselyn, S. A. (2007). Neuronal competition and selection during memory formation. *Science, 316*(5823), 457–460. https://doi.org/10.1126/science.1139438.

Howe, M. L. (2011). The Adaptive Nature of Memory and Its Illusions. *Current Directions in Psychological Science, 20*(5), 312–315. https://doi.org/10.1177/0963721411416571.

Hsiang, H. L., Epp, J. R., van den Oever, M. C., Yan, C., Rashid, A. J., Insel, N., ... Josselyn, S. A. (2014). Manipulating a "cocaine engram" in mice. *Journal of Neuroscience, 34*(42), 14115–14127. https://doi.org/10.1523/JNEUROSCI.3327-14.2014.

Hyman, I., Husband, T., & Billings, F. (1995). False Memories of Childhood Experiences. *Applied Cognitive Psychology, 9*, 181–197. https://doi.org/10.1002/acp.2350090302.

Josselyn, S. A., Köhler, S., & Frankland, P. W. (2015). Finding the engram. *Nature Reviews Neuroscience, 16*(9), 521–534. https://doi.org/10.1038/nrn4000.

Josselyn, S. A., Shi, C., Carlezon, W. A., Jr., Neve, R. L., Nestler, E. J., & Davis, M. (2001). Long-term memory is facilitated by cAMP response element-binding protein over-expression in the amygdala. *Journal of Neuroscience, 21*(7), 2404–2412.

Kawashima, T., Kitamura, K., Suzuki, K., Nonaka, M., Kamijo, S., Takemoto-Kimura, S., ... Bito, H. (2013). Functional labeling of neurons and their projections using the synthetic activity-dependent promoter E-SARE. *Nature Methods, 10*(9), 889–895. https://doi.org/10.1038/nmeth.2559.

Kurkela, K. A., & Dennis, N. A. (2016). Event-related fMRI studies of false memory: An Activation Likelihood Estimation meta-analysis. *Neuropsychologia, 81*, 149–167. https://doi.org/10.1016/j.neuropsychologia.2015.12.006.

Laney, C., & Loftus, E. F. (2013). Recent advances in false memory research. *South African Journal of Psychology, 43*(2), 137–146. https://doi.org/10.1177/0081246313484236.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: Sensory interface of the amygdala in fear conditioning. *Journal of Neuroscience, 10*(4), 1062–1069.

Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., & Tonegawa, S. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature, 484*(7394), 381–385. https://doi.org/10.1038/nature11028.

Loftus, E. F. (2003). Make-believe memories. *American Psychologist, 58*(11), 867–873. https://doi.org/10.1037/0003-066X.58.11.867.

Loftus, E. F. (2005). Planting misinformation in the human mind: A 30-year investigation of the malleability of memory. *Learning & Memory, 12*(4), 361–366. https://doi.org/10.1101/lm.94705.

Loftus, E. F., Miller, D. G., & Burns, H. J. (1978). Semantic integration of verbal information into a visual memory. *Journal of Experimental Psychology Human Learning, 4*(1), 19–31.

Loftus, E. F., & Pickrell, J. E. (1995). The formation of false memories. *Psychiatric Annals, 25*(12), 720–725. https://doi.org/10.3928/0048-5713-19951201-07.

Maren, S. (2005). Synaptic mechanisms of associative memory in the amygdala. *Neuron, 47*(6), 783–786. https://doi.org/10.1016/j.neuron.2005.08.009.

Maren, S., & Fanselow, M. S. (1996). The amygdala and fear conditioning: Has the nut been cracked? *Neuron, 16*(2), 237–240. https://doi.org/10.1016/s0896-6273(00)80041-0.

McNally, R. J., & Geraerts, E. (2009). A new solution to the recovered memory debate. *Perspectives on Psychological Science, 4*(2), 126–134. https://doi.org/10.1111/j.1745-6924.2009.01112.x.

Neve, R. L., Neve, K. A., Nestler, E. J., & Carlezon, W. A., Jr. (2005). Use of herpes virus amplicon vectors to study brain disorders. *BioTechniques, 39*(3), 381–391.

Park, A., Jacob, A. D., Walters, B. J., Park, S., Rashid, A. J., Jung, J. H., ... Josselyn, S. A. (2020). A time-dependent role for the transcription factor CREB in neuronal allocation to an engram underlying a fear memory revealed using a novel in vivo optogenetic tool to modulate CREB function. *Neuropsychopharmacology, 45*(6), 916–924. https://doi.org/10.1038/s41386-019-0588-0.

Park, S., Kramer, E. E., Mercaldo, V., Rashid, A. J., Insel, N., Frankland, P. W., & Josselyn, S. A. (2016). Neuronal Allocation to a Hippocampal Engram.

Neuropsychopharmacology. https://doi.org/10.1038/npp.2016.73.

Pavlov, I. (1927). *Conditioned reflexes.* Oxford, England: Oxford University Press.

Perkins, C. C., Jr, & Weyant, R. G. (1958). The interval between training and test trials as a determiner of the slope of generalization gradients. *Journal of Comparative and Physiological Psychology, 51*(5), 596–600. https://doi.org/10.1037/h0042550.

Pignatelli, M., Ryan, T. J., Roy, D. S., Lovett, C., Smith, L. M., Muralidhar, S., & Tonegawa, S. (2019). Engram cell excitability state determines the efficacy of memory retrieval. *Neuron, 101*(2), 274–284.e275. https://doi.org/10.1016/j.neuron.2018.11.029.

Ramirez, S., Liu, X., Lin, P. A., Suh, J., Pignatelli, M., Redondo, R. L., ... Tonegawa, S. (2013). Creating a false memory in the hippocampus. *Science, 341*(6144), 387–391. https://doi.org/10.1126/science.1239073.

Rashid, A. J., Yan, C., Mercaldo, V., Hsiang, H. L., Park, S., Cole, C. J., ... Josselyn, S. A. (2016). Competition between engrams influences fear memory formation and recall. *Science, 353*(6297), 383–387. https://doi.org/10.1126/science.aaf0594.

Roediger, H. L., & McDermott, K. B. (2000). Tricks of memory. *Current Directions in Psychological Science, 9*(4), 123–127. https://doi.org/10.1111/1467-8721.00075.

Ruediger, S., Vittori, C., Bednarek, E., Genoud, C., Strata, P., Sacchetti, B., & Caroni, P. (2011). Learning-related feedforward inhibitory connectivity growth required for memory precision. *Nature, 473*(7348), 514–518. https://doi.org/10.1038/nature09946.

Schacter, D. L. (1999). The seven sins of memory. Insights from psychology and cognitive neuroscience. *American Psychologist, 54*(3), 182–203. https://doi.org/10.1037//0003-066x.54.3.182.

Schacter, D. L. (2020). The Seven Sins of Memory: How the Mind Forgets and Remembers (Revised edition). Boston and New York: Houghton Mifflin (in press).

Schacter, D. L., Carpenter, A. C., Devitt, A. L., & Thakral, P. P. (2020). Memory errors and distortion. In M. J. Kahana & A. D. Wagner (Eds.), The Oxford Handbook of Human Memory. New York: Oxford University Press (in press).

Schacter, D. L., Guerin, S. A., & St Jacques, P. L. (2011). Memory distortion: An adaptive perspective. *Trends in Cognitive Sciences, 15*(10), 467–474. https://doi.org/10.1016/j.tics.2011.08.004.

Schlichting, M. L., & Frankland, P. W. (2017). Memory allocation and integration in rodents and humans. *Current Opinion in Behavioral Sciences, 17*, 90–98.

Schlichting, M. L., & Preston, A. R. (2015). Memory integration: Neural mechanisms and implications for behavior. *Current Opinion in Behavioral Sciences, 1*, 1–8. https://doi.org/10.1016/j.cobeha.2014.07.005.

Shaw, J., & Porter, S. (2015). Constructing rich false memories of committing crime. *Psychological Science, 26*(3), 291–301. https://doi.org/10.1177/0956797614562862.

Slotnick, S. D., & Schacter, D. L. (2004). A sensory signature that distinguishes true from false memories. *Nature Neuroscience, 7*(6), 664–672. https://doi.org/10.1038/nn1252.

Smalarz, L., & Wells, G. L. (2015). Contamination of Eyewitness Self-Reports and the Mistaken-Identification Problem. *Current Directions in Psychological Science, 24*(2), 120–124. https://doi.org/10.1177/0963721414554394.

Sørensen, A. T., Cooper, Y. A., Baratta, M. V., Weng, F.-J., Zhang, Y., Ramamoorthi, K., ... Lin, Y. (2016). A robust activity marking system for exploring active neuronal ensembles. *Elife, 5*, Article e13918. https://doi.org/10.7554/eLife.13918.

Stahlberg, M., Ramakrishnan, C., Willig, K., Boyden, E., Deisseroth, K., & Dean, C. (2019). Investigating the feasibility of channelrhodopsin variants for nanoscale optogenetics. *Neurophotonics, 6*(1), Article 015007.

Vetere, G., Tran, L. M., Moberg, S., Steadman, P. E., Restivo, L., Morrison, F. G., ... Frankland, P. W. (2019). Memory formation in the absence of experience. *Nature Neuroscience.* https://doi.org/10.1038/s41593-019-0389-0.

Watson, J. B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology, 3*(1), 1–14. https://doi.org/10.1037/h0069608.

Wells, G. L., & Olson, E. A. (2003). Eyewitness testimony. *Annual Review of Psychology, 54*, 277–295. https://doi.org/10.1146/annurev.psych.54.101601.145028.

Wiltgen, B. J., & Silva, A. J. (2007). Memory for context becomes less specific with time. *Learning & Memory, 14*(4), 313–317. https://doi.org/10.1101/lm.430907.

Yiu, A. P., Mercaldo, V., Yan, C., Richards, B., Rashid, A. J., Hsiang, H. L., ... Josselyn, S. A. (2014). Neurons are recruited to a memory trace based on relative neuronal excitability immediately before training. *Neuron, 83*(3), 722–735. https://doi.org/10.1016/j.neuron.2014.07.017.

Yokose, J., Okubo-Suzuki, R., Nomoto, M., Ohkawa, N., Nishizono, H., Suzuki, A., ... Inokuchi, K. (2017). Overlapping memory trace indispensable for linking, but not recalling, individual memories. *Science, 355*(6323), 398–403. https://doi.org/10.1126/science.aal2690.

Zhang, F., Aravanis, A. M., Adamantidis, A., de Lecea, L., & Deisseroth, K. (2007). Circuit-breakers: Optical technologies for probing neural signals and systems. *Nature Reviews Neuroscience, 8*(8), 577–581. https://doi.org/10.1038/nrn2192.

Zhou, Y., Won, J., Karlsson, M. G., Zhou, M., Rogerson, T., Balaji, J., ... Silva, A. J. (2009). CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. *Nature Neuroscience, 12*(11), 1438–1443. https://doi.org/10.1038/nn.2405.